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TITLE: Prostate Specific Antigen-Triggered Tripartate Prodrug of S-trityl-L-Cysteine, an Eg5 Kinesin Inhibitor and Antimitosis Agent with Low Neurotoxicity

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14. ABSTRACT

A tripartate prodrug of S-trityl-L-cysteine was synthesized and investigated for antiprostatic potency versus the free anticancer agent (S-trityl-L-cysteine) in PSA-secreting cells (LNCap) and non-PSA-secreting cells (PC3). The prodrug did not inhibit cell growth as much as S-trityl-L-cysteine itself, was not selective for PSA-secreting cells, and did not release S-trityl-L-cysteine when treated with cell-free PSA. Interestingly, S-trityl-L-cysteine itself selectively inhibited the growth of LNCap versus PC3 cells.

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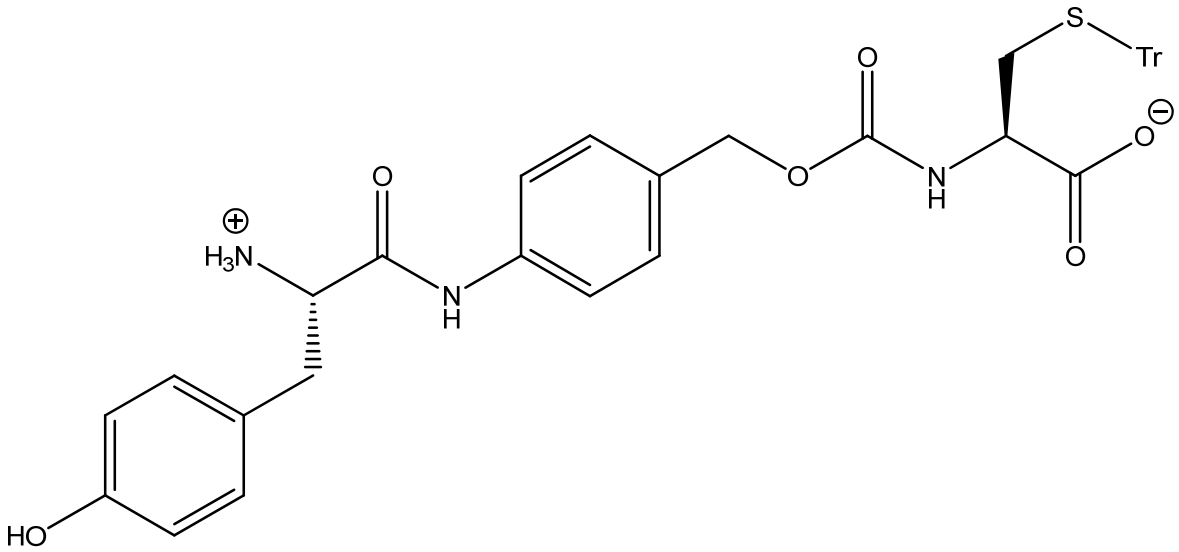
INTRODUCTION

The subject of this research was the synthesis and *in vitro* biological testing of a PSA-activated, antiprostatic prodrug with low neurotoxicity. The anticancer agent S-trityl-L-cysteine (STL or STLC) is the antiprostatic agent, which was coupled to a PSA-activated, self-releasing linker. The purpose of the project was to gather proof-of-principle data regarding selective *in vitro* cytotoxicity of the STLC prodrug towards PSA-secreting prostate cancer cells versus non-PSA-secreting cells. The scope of this project was limited to the synthesis of the proposed STLC prodrug and the investigaton of *in vitro* biological effects of STLC and the STLC prodrug.

BODY

Synthesis of an S-trityl-L-cysteine tripartate prodrug was completed. However, only the L-tyrosyl (Y) portion of the carrier was attached to the linker-drug assembly, formulating compound **1** (or RA).



1 or RA

The structure of compound **1** (or RA) was confirmed by proton and ^{13}C-NMR spectra. However, my colleague, Prof. Graham Jones at Northeastern University, was unable to complete the attachment of the HSSKL portion of the linker. Because we had a model compound which could still act as a PSA-activated prodrug, I decided to continue biological studies with the present compound.

We examined the ability of human PSA to release S-trityl-L-cysteine (STL) from RA under physiological conditions (pH 7.4, 37^{o}C). In our studies, even after 1 hr exposure to 1 mM RA, PSA was unable release S-trityl-L-cysteine, measured by TLC against S-trityl-L-cysteine standards . We concluded that, at least with the highest PSA concentrations that we could achieve (ca. 1 μM), RA is not an acceptable substrate for PSA. This may be due to the severe steric encumbrance of the trityl moiety in PSA's active active site.

RA was next sent to Dr. Glenn J. Bubley's lab at Harvard Medical School. His group examined the selectivity of RA versus STL itself against PSA-secreting prostate cancer cells and non-PSA-secreting cancer cells. Our hypothesis was that RA should be relatively inert unless activated by a serine protease (PSA) excreted by prostate cancer cells, thus activating the drug and releasing the STL cytotoxic component.

Two prostate cancer cell lines were used for these experiments: PC3, which does not produce or secrete PSA and is therefore a negative control, and LNCap, which produces and secretes PSA. Following treatment with STL and RA, cell proliferation was assessed by counting live cells.

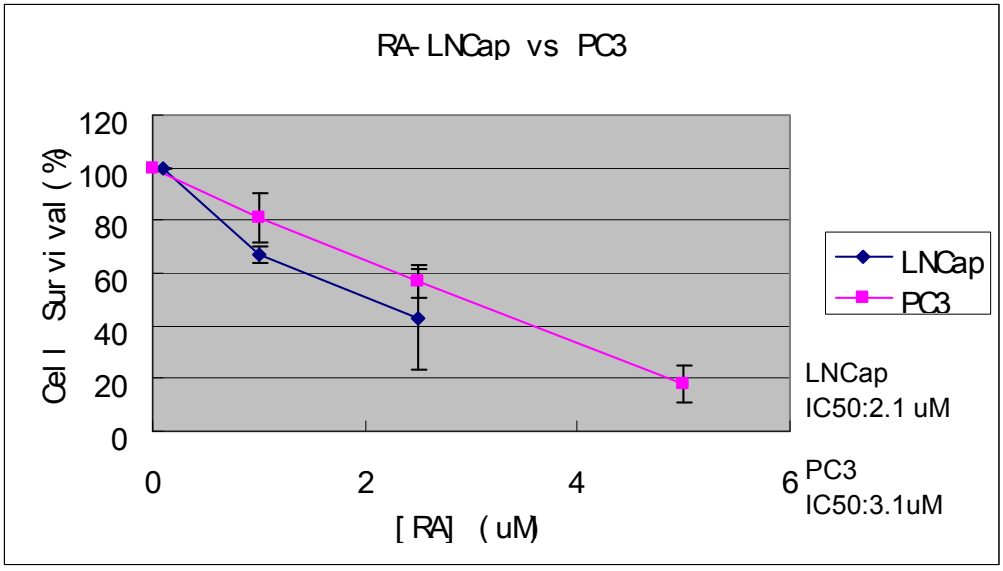


Figure 1: Percent cell survival of LNCap and PC3 cells exposed to RA (**1**).

Figure 1 compares the cytotoxicity of RA in LNCap (PSA excreting cells) and PC3 (non-PSA excreting cells) lines. The IC_{50} of LNCap cells is 2.1 μM and for PC3 cells is 3.1 μM.

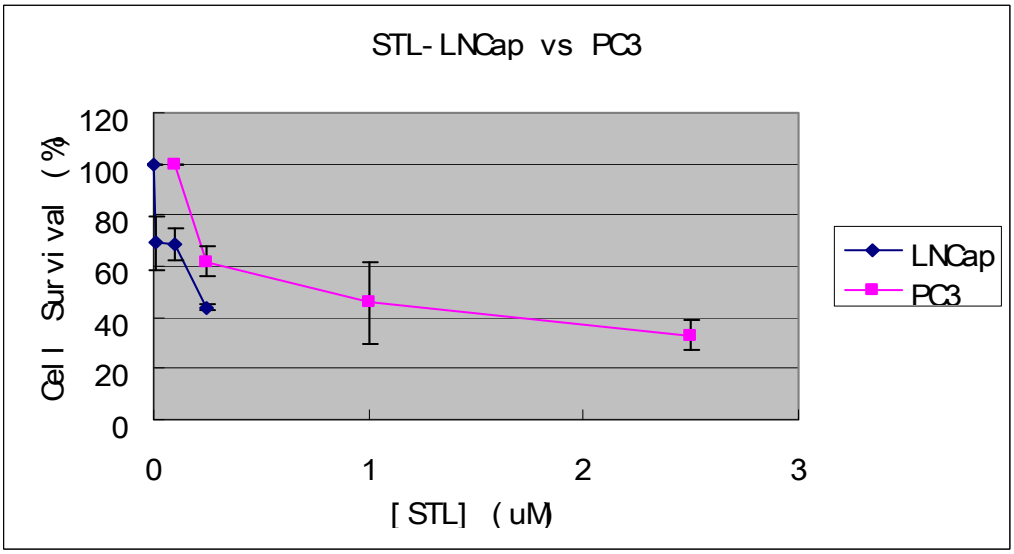


Figure 2. Percent cell survival of LNCAp and PC3 cells treated with STL (S-trityl-L-cysteine).

Figure 2 illustrates a marked difference in the cytotoxicity of STL on LNCap versus PC3 cell lines. STL IC_{50} for PC3 cells :0.95 μM and LNCap :0.176 μM.

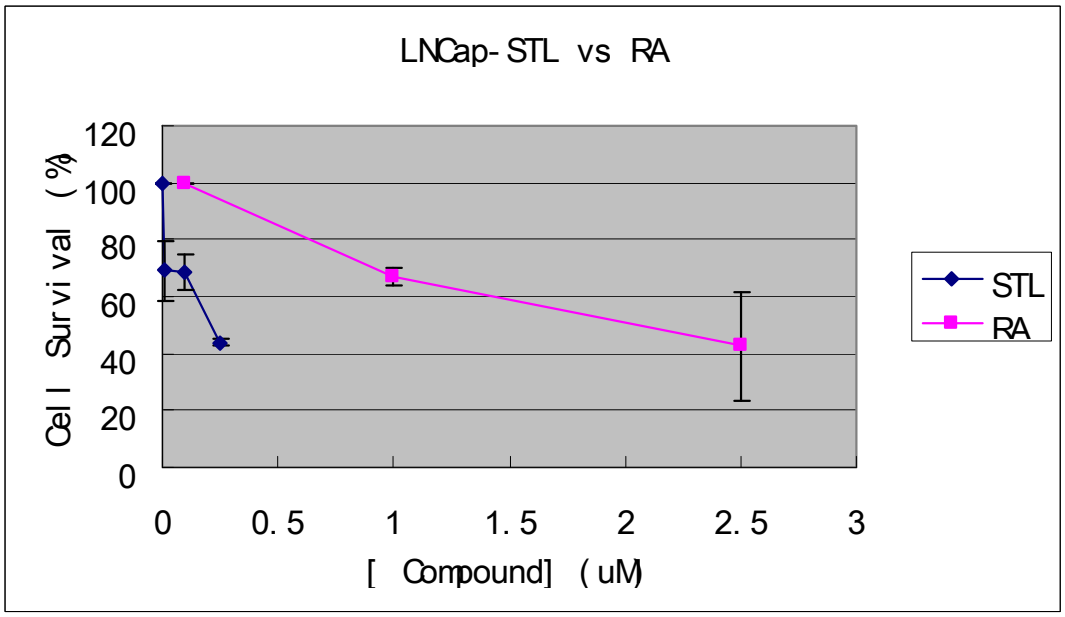


Figure 3. Percent cell survival of LNCap cells treated with either STL (S-trityl-L-cysteine) or RA (1).

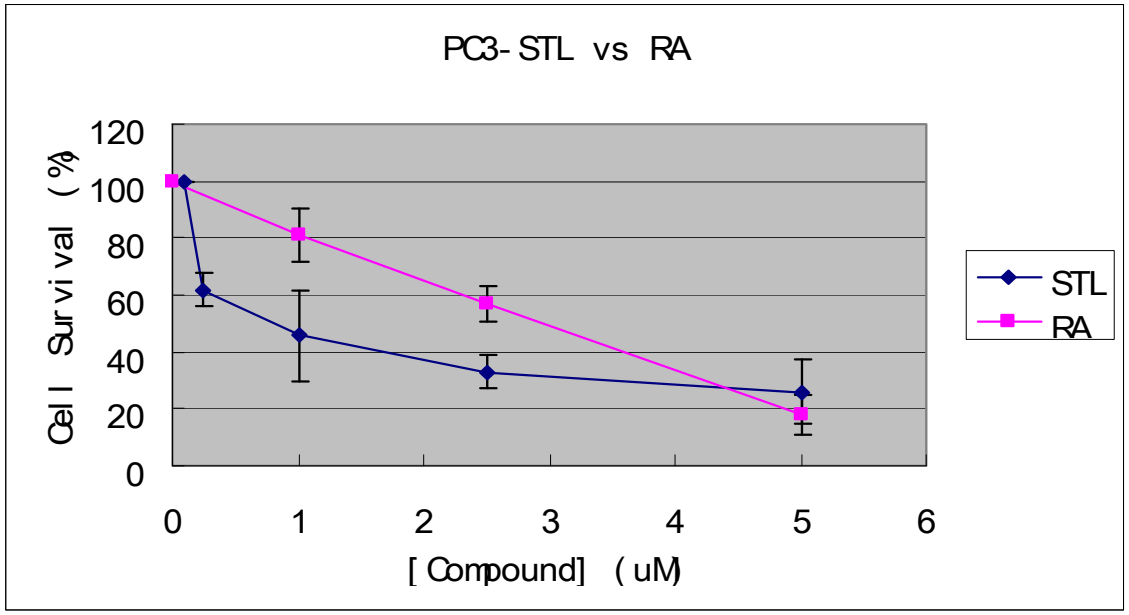


Figure 4. Percent cell survival of PC3 cells treated with either STL (S-trityl-L-cysteine) or RA (1).

In comparison to PC3 cells, LNCap cell lines were particularly affected by STL and RA. As seen in Figures 1 and 2, LNCap cells were slightly more sensitive to RA and more than fivefold more sensitive to STL than PC3 cells. Figure 3 also indicates that LNCap cells were also more sensitive to STL than RA.

Figures 3 and 4 compare the cytotoxicity of STL and RA against PC3 and LNCap cells, respectively. In both cell lines, STL had a more dramatic effect than RA. Towards LNCap cells (Fig. 3), STL produced an IC_{50} of 0.175 μM versus only 2.1 μM for RA. Against the PC3 cell line (Fig. 4), STLC gave an IC_{50} of 0.95 μM versus 3.1 μM for RA.

KEY RESEARCH ACCOMPLISHMENTS

- Synthesized a tripartate prodrug of S-trityl-L-cysteine (STL), designating the prodrug as RA.
- Analyzed RA as a substrate for cell-free PSA under optimum conditions.
- Compared the cytotoxicity of RA and STL towards both PSA-secreting and non-PSA-secreting prostate cancer cells.

REPORTABLE OUTCOMES

Presentations by undergraduate research assistants, National Council of Undergraduate Research, Salisbury University, 4/10/08-4/12/08:

(1) Oral session 6: Synthesis of tripartate prodrug and antiprostatic testing with PSA secreting and non-PSA-secreting cell lines. Presenter: Ms. Rachel Marine.
(2) Poster session 8: Biological study of S-trityl-L-cysteine and chemical analysis of S-trityl-L-cysteine and its prodrug. Presenter: Ms. Shakila Ziashakeri.

CONCLUSIONS

The purpose of these experiments was to determine if RA is selectively cytotoxic to LNCaP (PSA secreting cells) compared to PC-3 (non-PSA secreting cells). If we compare the ratios of IC_{50}STL/ IC_{50}RA in LNCap and PC3 cells, it will provide information as to whether or not RA is selectively cleaved by PSA in LNCap cells. A ratio close to 1 would indicate nearly complete selective cleavage of RA in LNCap cells. The STL/RA ratio of the IC50 values for **LNCap is 0.175/2.1=0.083 and for PC3 is 0.95/3.1=0.31**. According to this data, PC3 cells have a ratio closer to 1 compared to LNCap cells. Therefore this experiment refutes the hypothesis that RA is selectively cytotoxic towards PSA-secreting cells.

This result forces us to question whether RA is significantly cleaved by PSA. HPLC or GC/MS data of conditioned media and RA solution may be of interest to determine if RA compound is in fact cleaved by PSA to release the toxic STL component. Another reason for a negative result might be that the PSA secreted into the media is rapidly and relatively completely protein-bound, making this relatively inert as a serine protease. This second reason is unlikely, because in previous experiments of PSA-cleavable prodrugs, the LNCap cell line and the growth medium conditions did not adversely affect drug release.[1,2]

The data suggests that LNCap cells, compared to PC3, cells are more responsive to the STL active drug. Perhaps LNCap cells have a different uptake mechanism compared to PC3 cells for this Eg5 inhibitor. It may be possible to optimize this selective cytotoxicity of STL through chemical modification and to create a targeted antiprostatic agent that has no need for PSA activation. A starting point may be the more potent analogs of STL recently reported in the literature.[3]

We have determined that RA is not completely/selectively cleaved by PSA excreted by LNCap cells. Our experiment demonstrating the inability of cell-free PSA to cleave RA also supports this conclusion. Surprisingly, we have also discovered that STL itself is five-fold more cytotoxic towards LNCap than PC3 cells.

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